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Investigations on some metabolites of *Tecoma stans* Juss. callus tissue

Part III. Chromatographical search for iridoids, phenolic acids, terpenoids and sugars

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Abstract

Tissue cultures of *Tecoma stans* Juss. cultivated on modified Murashige-Skoog medium (RT-k) were phytochemically analysed by means of chromatographical methods (PC, TLC). The following products were found as metabolites: phenolic acids — chlorogenics, caffeic, ferulic, vanillic, o-coumaric and sinapic; steroids — β-sitosterol; triterpenes — ursolic and oleanolic acids, α-amyrine; sugars — glucose, fructose, sucrose, xylose. Meso-inositol was isolated in 0.8% yield. In intact plant leaves, some differences concerning the content and/or number of individual compounds were observed, namely: lack of sinapic acid and occurrence of p-coumaric acid, lower content of β-sitosterol, lack of oleanolic acid, occurrence of β-amyrine and of one unidentified triterpenoid, lack of xylose, occurrence of maltose, raffinose, and stachiose. The level of meso-inositol in leaves was distincly lower than in the callus tissues. Neither in callus tissues nor in leaves iridoid glycosides were found.

INTRODUCTION

Monoterpenoid alkaloids, present in *Tecoma stans* Juss., originate probably in iridoid compounds and are formed by the replacement of heterocyclic oxygen by nitrogen (Gross, Berg, Schütte, 1972). The existence of such biogenetic pathways in numerous species is confirmed by the occurrence of monoterpenoid alkaloids accompanied by iridoid compounds of analogous structure, such as: actinidine and matatabilactone in *Actinidia polygama* Sieb. et Zucc. (Sakan, Fujino, Murai, Butsugan, Suzui, 1960), boschniakine and boschnialactone in *Boschniakia rossica* Hult. (Sakan, Murai, Hayashi, Honda, Shono, Nakaj-

jima, Kato, 1967), methoxyactinidine and valtrates in *Valeriana officinalis* L., and monoterpenoid alkaloids, indolic alkaloids, and asperuloside in *Alstonia* sp. (Gross, 1970).

Since the presence of monoterpenoid alkaloids characteristic for the intact plant has been observed in tissue culture of *T. stans* (Dohnal, 1976 b), it should be expected that, in the plant itself as well as in callus tissue, these compounds might be accompanied by some iridoid glycosides, found in the *Bignoniaceae* family, e.g.: catalposide in the *Catalpa* genus (Hegnauer, 1964), 6-0-veratrylcatalposide in *Tecomella undulata* G. Don. (Joshi, Prakash, Singh, 1975), amorphous iridoid glycoside in *Tecoma capensis* Lindl. (Hammouda, Khalafaliah, 1971). The latter compound is an ester of the trans-p-methoxycinnamic acid. It has been isolated from the leaves of *Tecoma fulva* (Cav.) G. Don., along with monoterpenoid alkaloids (Hilz, Edelmann, Appel, 1973).

In the *Bignoniaceae* family, numerous aromatic hydroxyacids have been discovered, such as: chlorogenic, caffeic, ferulic, sinapic, and vanillic and esters of benzoic and cinnamic acids (Hegnauer, 1964).

It seemed purposeful, therefore, to examine both the intact plant and the callus tissue of *T. stans* for the presence of iridoid glycosides as well as phenolic acids. For a more complete comparison of metabolism of tissues grown *in vitro* and *in vivo,* the levels of certain sugars, steroids, and triterpenoids in callus tissues and leaves of greenhouse plants were determined.

MATERIAL AND METHODS

Callus tissues grown on modified Murashige-Skoog liquid culture medium, RT-k (Dohnal, 1976 a), and the leaves of 2—3 year old greenhouse plants were the starting material for examinations. Iridoid glycosides, phenolic acids, and sugars were determined in homogenized fresh tissues, steroids and triterpenoids in dried (50—60° C) material.

To search for iridoid glycosides, extracts were prepared by Trim-Hill method (Trim, 1951; Trim, Hill, 1951): 50 g of fresh tissue was refluxed on the boiling water bath, with 100 ml of distilled water and 120 mg of calcium carbonate, for 3 hours. After cooling the mixture was filtered and the filtrate was stirred with the charcoal then filtered off again, this time through the kieselguhr layer and washed with distilled water followed by 50% ethanol. The alcohol filtrate (glycoside fraction) was reduced under lowered pressure to 10 ml (extract B).

For phenolic acid determination, extracts were prepared by the Bate-Smith method (1961) : 2 g of tissue was refluxed on boilingwater bath with 10 ml of 2N hydrochloric acid for 20 minutes, and filtered after cooling. The filtrate was shaken with 0,5 ml of amyl alcohol (extract C).

Steroids and triterpenoids were identified in an acidic chloroform extract A, obtained by extraction of plant material according to Dickinson-Jones method (extraction scheme — Dohnal, 1976 b).

Tissue extracts for estimation of free simple sugars and their derivatives were prepared by the alcohol extraction method (Supniewska, Dohnal, 1972) : 5 g of tissue was homogenized with 4 ml of ethyl alcohol and decanted after centrifugal separation, obtaining ab. 4 ml of liquid (extract D).

The obtained extracts were studied by the paper (PG) and thin layer (TLC) chromatography methods, using the following adsorbents and solvent systems:

a) Whatmann paper No. 1, descending method, path length 45 cm, system n-butanol-acetic acid-dist. water (4 : 1 : 5v/v), organic phase, development time 18—20 h (Friedrich, Schönert, 1973; Partridge, 1948);

b) Whatmann paper No. MM-3, ascending method, path length 30 cm, system n-butanol-acetic acid-dist. water (4 : 1 : 2 v/v), development time 17 h, (Hedin, Minyard, Thompson, 1967; Oświecimska, Sendra, 1972);

c) Whatmann paper No. 1, descending method, path length 42 cm, system ethyl acetate-n-butanol-acetic acid-dist. water (3 : 4 : 2,5 : 4 v/v), development time 17—20 h (Imhoff, Bourdu, 1973);

d) Whatmann paper No. 1, desceding method, path length 42 cm, system: acetone-dist. water (85 : 15 v/v), development time 17 h (Imhoff, Bourdu, 1973);

e) silica gel G (E. Merck), cellulose powder MN 300 (Macherey, Nagel Co) 1 : 1, system: toluene-butyl acetate-80% formic acid (5 : 5 : 3 v/v), development time 40 min (Grodzińska-Zachwieja, Kahl, Klimczak, 1971);

f) sorbent as in e, system: toluene-ethyl formate-80% formic acid (5 : 4 : 1 v/v), development time 50 min (Van Sumere, Wolf, Teuchy, Kint, 1965);

g) silica gel G (E. Merck), system: benzene-methanol-acetic acid (45 : 8 : 4 v/v), time of development 1 h (Hedin, Minyard, Thompson, 1967);

h) silica gel (E. Merck), system: benzene-ethyl acetate-acetic acid (90 : 10 : 1 v/v), development time 1 h (Dudley, Chiang, 1969);

i) silica gel H (E. Merck), system: n-hexane-chloroform-ethyl acetate (4 : 1 : 1 v/v), development time 40—60 min. (Tomita, Uomori, Minato, 1969);

j) silica gel H (E. Merck), system: chloroform-petroleum ether 40—60° C (90 : 10 v/v), development time 1,5 h, (Benjamin, Mulchandani, 1973);

k) silica gel, "Silufol" UV 254 plates Serva, system: chloroform-methanol-dist. water (64 : 36 : 8 v/v), development time 1 h (Karting, Wegscheider, 1972);

l) sorbent as in k, system: ethyl acetate-methanol-acetic acid-dist. water (60 : 15 : 15 : 10 v/v), development time 1 h, (Kartnig, Wegscheider 1972). Path length in thin layer chromatography was 17 cm.

Iridoid glycosides were studied according to:

a) Trim-Hill method (1951) — 1 ml of tissue extract was heated for 5 min. with a reagent prepared by mixing 10 ml glacial acetic acid, 1 ml of 0,2% solution of copper sulphate, and 0,5 ml of concentrated hydrochloric acid. (Iridoid glycosides produce, under these conditions, colorful decomposition products, e.g. blue — asperuloside);

b) the paper chromatography method in the solvent system a. Developed chromatograms were sprayed with the Trim-Hill reagent (see above), then heater for 5 min. (100° C), or with a 1% solution of vaniline in methanol, then subjecting it to vapors of concentrated hydrochloric acid (Trim, Hill, 1951). Asperuloside of the K and K Laboratories Inc., Jamaica, N. Y. was used as the reference substance.

Phenolic acids were studied chromatographically in four solvent systems: b, e, f, g. 50 μl of extract corresponding to 200 mg of fresh tissue, was spotted along the starting line (1 cm in length). As standards were used 1% methanol solutions of the following phenolic acids: caffeic, chlorogenic, isochlorogenic, ferulic, isoferulic, vanillic, sinapic, o-, m-, and p-coumaric, of which 2—5 μl (20—50 γ) were applied*.

Chromatograms were air dried and then examined under UV light before and after being treated with ammonia vapors. The following reagents were used for visualisation: 2% methanol solution of iron chloride (Turowska, Kohlmünzer, Molik-Węgiel, 1970); the Benedict reagent (Stahl, 1969) followed by Pauly's reagent (Stahl, 1969), what resulted in a better differentiation of colours; 1% solution of potassium permanganate (Stahl, 1969), for detecting o-, m-, and p-methoxycinnamic acids.

Steroids and triterpenoids were identified by the chromatographic methods (PC and TLC) in solvent systems a, h, i, j. Extracts were purified by the thin layer preparative chromatography method (Kaul, Staba, 1968), system h, the adsorbent layer 1 mm thick. Steroid and triterpenoid bands were marked (R_f values 0.1—0.2 and 0.4—0.6), scraped along and exhaustively eluated with chloroform. The concentrated chloroform extracts were chromatographed along with the standard substances: β-sitosterol, stigmasterol, β- and α-amyrine, oleanolic and ursolic acids, spotting at the starting points quantities of tissue extracts corresponding to ab.

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2—5 g of dry callus tissue and 0.2—0.3 g of dry leaves. Developed chromatograms were examined under UV light, and sprayed with the following reagents: 15% $SbCl_3$ chloroform solution, 20% $SbCl_5$ carbon tetrachloride solution, Liebermann-Burchard (Stahl, 1969).

Simple sugars and their derivatives were chromatographically examined in 5 solvent systems a, c, d, k, l. In order to obtain a better separation of spots, in system a, continuous chromatography has been employed (42—44 h). Extracts amounts corresponding to 100 mg of fresh callus tissue, or 50 mg of fresh leaves, were spotted along the starting line. The following standards were used: arabinose, xylose, ribose, rhamnose, glucose, galactose, fructose, sucrose, lactose, maltose, raffinose, stachiose, and meso-inositol. For sugar visualisation aniline oxalate was used (Partridge, 1948), as well as resorcine in hydrochloric acid (Supniewska, Dohnal, 1972), thymol in sulphuric acid (Kartnig, Wegscheider, 1972; Stahl, 1969), and for meso-inositol the Tollens reagent (Stahl, 1969). Sprayed chromatograms were dried (100—110° C) up to the moment of appearance of distinc spots (10—20 min.).

The isolated meso-inositol was purified by recrystallisation from 50% methanol, the melting point and the IR spectrum (KBr, Unicam SP 200) were determined, comparing them with the same standard substance datas.

RESULTS AND DISCUSSION

No iridoid glycosides, even in trace quantities, were found in callus tissues and leaves, using the above mentioned methods.

In chromatographic study (PC, TLC) extracts C showed some spots characteristic for phenolic derivatives. On the basis of the size and intensity of spots, conclusions were drawn as to the quantities of the present compounds (Fig. 1, Table 1).

In callus tissues the significant amounts of chlorogenics, caffeic, and ferulic acids were observed, smaller amounts of vanillic and sinapic acids, while only traces of o-coumaric acid.

In the leaves there were also large amounts of chlorogenics and caffeic acids, but in comparison to the callus tissue, significant differences as to the amounts and proportions of cinnamic and benzoic acid derivatives were observed. Thus, no sinapic acid was found, while the ferulic and vanillic acids were present in much smaller amounts. On the other hand the p-coumaric acid spot is dominant in leaves and the o-coumaric acid is also present in larger amounts than in callus tissues.

In the more selective solvent system f, five unidentified spots were observed, including three which were common for the callus and the leaf tissues. The R^f values of these spots, as well as their colours under UV light and with reagents, are listed in Table 1. These spots give reactions

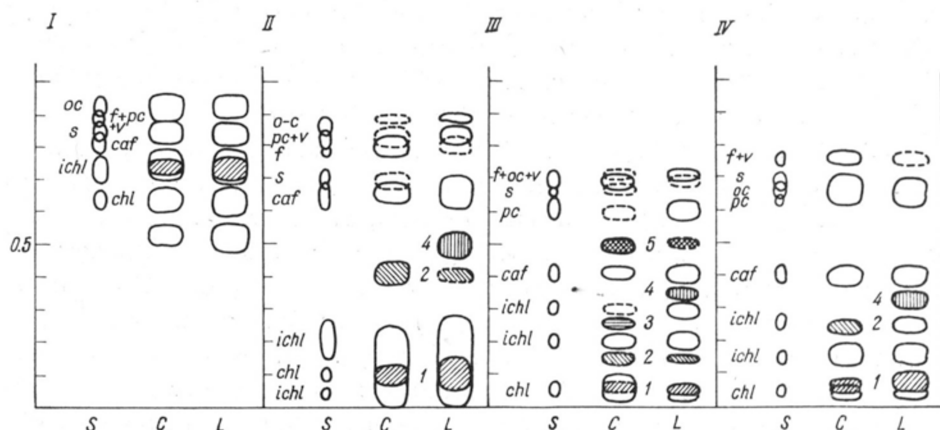

Fig. 1. Chromatograms of phenolic compounds (extracts C)

I, II, III, IV — solvent systems b, e, f, g, respectively; S — phenolic acid standards: *caf* — caffeic, *chl* — chlorogenic, *ichl* — isochlorogenic, *oc* — o-coumaric, *pc* — p-coumaric, *f* — ferulic, *s* — sinapic, *v* — vanillic; C — callus tissue extract; L — leaf extract. Hatched spots (1—5) — unidentified compounds; spots encircled with: continuous line — well marked, broken line — traces.

Table 1

Paper and thin-layer chromatography of phenolic compounds extracted from *Tecoma stans* callus tissues and leaves

Compounds	R_f values in solvent systems				Fluorescence		Colour reactions with reagents			Extracts C	
	b	e	f	g	UV	UV/ NH_3	$FeCl_3$	Benedict	Pauly	Callus tissues	Leaves
Phenolic acids											
iso-chloro-	0.72	0.05	0.20	0.15	bl	g-y	d bl-g	y	y	+++	+++
genic		0.20	0.29	0.25	,,	,,	,,	,,	,,	++	+++
chlorogenic	0.62	0.10	0.05	0.03	bl	g-y	d bl-g	y	y	+++	+++
caffeic	0.80	0.64	0.40	0.40	bl	b bl	d bl-g	y	ol	+++	+++
ferulic	0.87	0.80	0.69	0.73	bl	bl	—	sl	v	++	+
o-coumaric	0.91	0.84	0.67	0.70	p bl	p g	—	y	or	(+)	+
p-coumaric	0.89	0.82	0.60	0.65	d v	sp	—	—	r	—	++
sinapic	0.84	0.72	0.65	0.66	d v	sp	d bl-g	y	sl	+	—
vannillic	0.88	0.81	0.70	0.72	d v	sp-v	—	p y	or	+	(+)
Unidentified											
1.	0.73	0.10	0.05	0.07	bl	b bl	d bl-g	y	or	++	+++
2.	—	0.40	0.15	0.23	sp	bl	g-d bl	y	r-v	++	(+)
3.	—	—	0.24	—	p bl	—	—	—	r-or	++	—
4.	—	0.48	0.33	0.32	br	ol-y	d bl-g	d y	or	—	++
5.	—	—	0.50	—	sp	bl	d bl	y	r-or	++	(+)

Explanations:

Solvent systems b,e,f,g, — see in text; Colours: bl-blue, br-brown, g-green, ol-olive, or-orange, r-red, sl-salmony, sp-saphyre, v-violet, y-yellow, b-bright, d-dark, p-pale; Sings: +++ very large amounts, ++ considerable amounts, + well marked, (+) traces, — lack of compound.

characteristic for phenolic compounds. Spot No. 4 occuring only in the leaf extract could be considered as a flavonoid.

In extracts C of the callus tissue and leaves p-methoxycinnamic acid was not found, though this compound has been observed in some *Tecoma species* (Hammouda, Khalafaliah, 1971; Hilz, Edelmann, Appel, 1973) in free form or esterifying the iridoid glycosides.

The presence of significant amounts of phenolic acids is a proof of an intensive fundamental metabolism, characteristic for rapidly propagating tissue, while secondary metabolism pathways, leading to the biosynthesis of more complex compounds, were blocked (Feucht, 1973).

Steroid and triterpenoid compounds were identified in extracts A.

The results, averages of several experiments, are presented in Fig. 2.

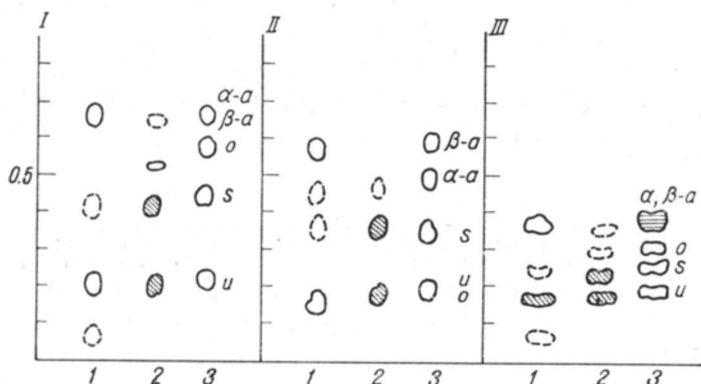

Fig. 2. Chromatograms of steroid and triterpenoid compounds (extracts A)

I, II, III — solvent systems i, j, h, respectively; 1 and 2 — leaf and callus tissue extracts, resp.; 3 — standards: αa — α-amyrine, βa — β-amyrine, o — oleanolic acid, s — β-sitosterol, u — ursolic acid; Hatched spots — very well marked, continuous line — well marked, broken line — traces.

In the callus tissue extract β-sitosterol, ursolic acid, and trace amounts of oleanolic acid and amyrine were found (Fig. 2, pt. 2). A comparison of the R_f values for β-amyrine (0.59) and α-amyrine (0.49) in the system j, allows to identify the spot R_f 0.48, appearing in this extract, as α-amyrine. The leaf extract contains also β-sitosterol, ursolic acid, and α-amyrine, moreover β-amyrine and an unidentified compound of a triterpenoid character (Fig. 2, pt. 1).

Steroids and triterpenoids occur commonly in the plant kingdom, either in unattached form, or ester, or glycosidic form. They have also been identified in tissue cultures which have preserved the ability to synthesize secondary metabolites, characteristic of the intact plant (Benjamin, Mulchandani, 1973; Benveniste, Hirth, Ourisson, 1966; Kaul, Staba, 1968; Khanna, Mohan, 1973; Staba, 1969;

Supniewska, Dohnal, 1972; Teuscher, 1973; Tomita, Uomori, Minato, 1969; Yanagawa, Kato, Kitahara, 1972).

When comparing the results obtained for callus tissues and leaves of *T. stans* following conclusion can be made: in leaf extracts the triterpene substances are dominant (4 spots) and the sterols are present only in traces (1 weak spot), whereas in callus tissue extracts the triterpene spectrum is distincly limited, on the other hand the β-sitosterol spot is more intensive. This observation is in agreement with the data concerning higher content of sterols in tissues growing *in vitro*, than in the intact plants, e.g. in: *Oryza sativa* L. (Yanagawa, Kato, Kitahara, 1972), *Momordica charantia* L. (Khanna, Mohan, 1973), *Solanum laciniatum* Ait. (Supniewska, Dohnal, 1972), and *Solanum xanthocarpum* Schard. et Wendl. (Teuscher, 1973).

In extracts D of callus tissue, 4 spots have been identified, coresponding to glucose, fructose, sucrose, and xylose. In the leaves, beside glucose, fructose, and sucrose, there were also maltose, raffinose, and stachiose, while no xylose was discovered, even in trace amounts. Moreover, unidentified spots of sugar character were also observed (Fig. 3).

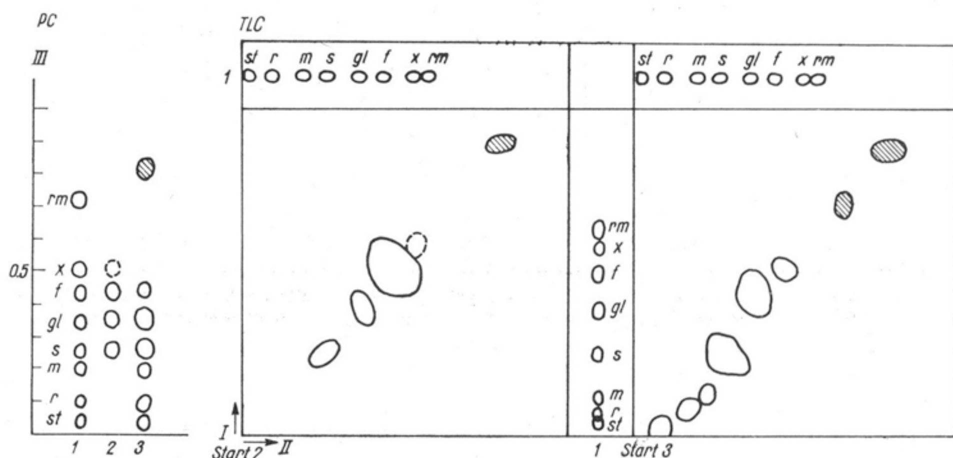

Fig. 3. One and two-dimensional chromatograms of sugars (extracts D) PC — paper chromatography, TLC — thin-layer chromatography

I, II, III — solvent systems k, l, a, resp.; 1 — standards: *f* — fructose, *gl* — glucose, *m* — maltose, *r* — raffinose, *rm* — rhamnose, *s* — sucrose, *st* — stachiose, *x* — xylose; 2 and 3 — callus tissue and leaf extracts, resp.; Hatched spots — unidentified compounds, spots encircled with: continuous line — well marked, broken line — traces.

In comparing the sizes and intensities of the chromatographic spots, differences were observed in the amounts of sugars present. In callus tissues glucose and fructose were predominant while less amounts of sucrose and traces of xylose. In the leaves glucose and sucrose were predominant and the remaining sugars were found in smaller amounts.

The poorer chromatographic spectrum of sugars in the callus tissue and the predominance of simple sugars over the complex ones reflects the altered metabolism of *in vitro* culture, where tissue is not fully autotrophic, being supplied with the basic assimilation products (sucrose or other sugars) in the culture medium. The consumption of these compounds by the cultivated tissues is markedly accelerated by the auxines added to the medium, which stimulating the cell divisions, counteract the normal tendency of cells to synthesize and store the more complex sugars. Even the storage tissue (endosperm), when cultivated *in vitro,* loses its ability to store starch, using up produced stores as the medium becomes exhausted (Mares, Stone, 1973; Meryl-Smith, Stone, 1973).

In extracts D, there has been observed also the presence of meso-inositol, whose R_f values correspond to the data for the standard substance: 0.1 (system a), 0.28 (system k), 0.7 (system l). Identical R_f values in those solvent systems were also characteristic for the compound obtained in crystalline form from crude methanol extract (Dohnal, 1976 b) of callus tissue, 0.8% yield as small white needles, m.p. 229° C (230° C, literature, Fa-Ching, Juh-Meei, See, 1972). The IR spectrum in KBr is identical with that of the meso-inositol standard, the absorbtion wave lengths: 3420, 3240 (OH), 2930 (CH) cm^{-1} (Fig. 4 a, b).

The much higher meso-inositol content in tissues grown *in vitro* than in leaves, may be the result of high level of this compound in the culture

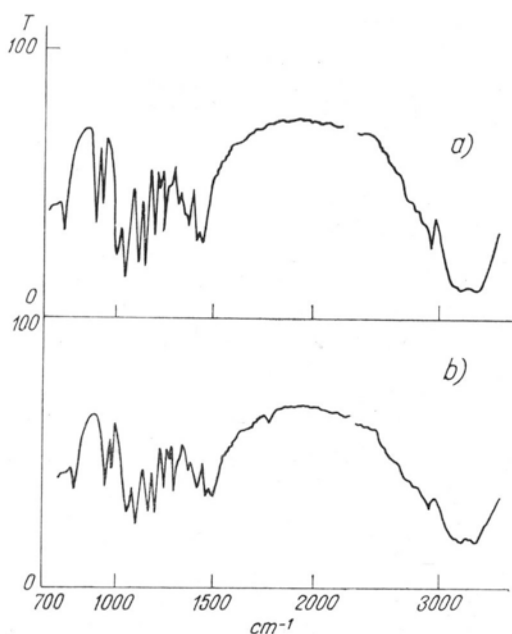

Fig. 4. IR spectrum of meso-inositol: a — original substance, b — compound isolated from *Tecoma stans* callus tissues

medium and is perhaps related to the increase in cell wall permeability and the active participation of meso-inositol in sugar and aminoacids membrane transport (Wood, Braun, 1961; Jung, Tanner, Wolter, 1972). Meso-inositol accumulation in callus tissues was also observed in tissue cultures of other species, e.g. *Vinca rosea* L. (own observation).

A comparison of compounds detected in callus tissues and leaves of *T. stans* presented in the part III of this work is shown in Table 2.

Table 2

Results of chromatographical analysis of steroids, triterpenoids, phenolics and sugars in *Tecoma stans* leaves and callus tissues

Extract	Identified compounds		Leaves	Callus tissues
A	STEROIDS	β-sitosterol	(+)	++
	TRITERPENOIDS	α-amyrine	+	(+)
		β-amyrine	++	—
		Oleanolic acid	—	(+)
		Ursolic acid	++	++
		Unidentifield	+	—
C	PHENOLICS	Caffeic acid	++	++
		o-Coumaric acid	+	(+)
		p-Coumaric acid	++	—
		Chlorogenic acids	++	++
		Ferulic acid	+	++
		Sinapic acid	—	+
		Vanillic acid	(+)	+
		Unidentified compds	++	++
		Flavonoid subst.	+	—
D	SUGARS	Fructose	+	++
		Glucose	++	++
		Maltose	+	—
		Raffinose	+	—
		Sucrose	++	+
		Stachiose	+	—
		Xylose	—	(+)
		meso-Inositol	+	++
		Unidentified	+	+

Explanations: ++ very large amounts, + considerable amounts, (+) traces, — lack of compound.

Summing up the total research work on callus tissue of *Tecoma. stans* Juss. (Dohnal, 1976a, b) the following conclusions may be presented:

1) The morphological and anatomical structure of tissue is considerably influenced by the composition of culture medium. The concentration of growth substances, sugar, and mineral salts, particularly nitrogen compounds are of essential importance.

2) There is a relationship between the organisational level of callus tissue and its ability for biosynthesis of secondary metabolism products.

3) Callus tissue of *T. stans* is capable to biosynthesise monoterpenoid alkaloids characteristic of this species, and quinone — lapachol as well as other primary and secondary metabolites such as sugars, phenolic acids, steroids, and triterpenoids. Differences were observed, however, in the numbers and leveles of these compounds as compared to the leaves of intact plants.

4) Quinolinic acid added to the culture medium had a stimulating effect on the alkaloid biosynthesis of *Tecoma*.

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REFERENCES

Bate-Smith E. C., 1961. J. Linsi. Soc. (Bot.), 58: 95.
Benjamin B. D., Mulchandani N. B., 1973. Planta Medica 23: 394.
Benveniste P., Hirth L., Ourisson G., 1966. Phytochemistry 5: 31.
Dohnal B., 1976 a. Acta Soc. Bot. Pol. p. I. 43 (1—2): 93.
Dohnal B., 1976 b. Acta Soc. Bot. Pol. p. II 43 (4): 369.
Dudley K. H., Chiang R. W., 1969. J. Org. Chem., 34: 120.
Fa-Ching Chen, Juh-Meei Lin, See Lin, 1972. Phytochemistry 11: 1496.
Feucht W., 1973. Mitt. Klosterneuburg 23. 131.
Friedrich H. Schönert J., 1973. Planta Medica 24: 90.
Grodzińska-Zachwieja Z., Kahl W., Klimczak M., 1971. Dissert. Pharm. Pharmacol. 23: 179.
Gross D., 1970. Fortschr. Bot. 32: 93.
Gross D., Berg W., Schütte H. R., 1972. Biochem. Physiol. Pflanzen 163: 576.
Hammouda Y., Khalafaliah N., 1971. Pharmazie 26: 640.
Hedin P. A., Minyard J. P., Thompson A. C., 1967. J. Chromat. 30: 43.
Hegnauer R., 1964. Chemotaxonomie der Pflanzen, Vol. III, Birkhäuser Verlag, Basel, p. 268—281.
Hilz W., Edelmann G., Appel H. H., 1973. Rev Lar. Quim. 4: 28.
Imhoff V., Bourdu R., 1973. Phytochemistry. 12: 331.
Joshi K. C., Prakash L., Singh L. B., 1975. Phytochemistry 14: 1441.
Jung P., Tanner W., Wolter K., 1972. Phytochemistry 11: 1655.
Kartnig Th., Wegscheider O., 1972. Planta Medica 21: 144.
Kaul B., Staba E. J., 1968. Lloydia, 31: 171.
Khanna P., Mohan S., 1973. J. Exp. Biol. 11: 58.
Mares D. J., Stone B. A., 1973. Aust. J. Biol. Sci. 26: 135.
Meryl Smith M., Stone B. A., 1973. Aust. J. Biol. Sci. 26: 123.
Oświecimska M., Sendra J., 1972. Dissert. Pharm. Pharmacol. 24: 475.
Partridge S. M., 1948. Biochem., J. 42: 238.
Sakan T., Fujino A., Murai F., Butsugan Y., Suzui A., 1960. C. A. 3484 g.

Sakan T., Murai F., Hayashi Y., Honda Y., Shono T., Nakajima M., Kato M., 1967. Tetrahedron 23: 4635.
Staba E. J., 1969. Recent Advances in Phytochemistry 2: 75.
Stahl E., 1969. Thin-Layer Chromatigraphy, Springer Verlag, Berlin.
Supniewska J. H., Dohnal B., 1972. Dissert. Pharm. Pharmacol. 24: 193.
Teuscher E., 1973. Pharmazie 28: 6.
Tomita Y., Uomori A., Minato H., 1969. Phytochemistry 8: 2249.
Trim A. R., 1951. Biochem J. 50: 319.
Trim A. R., Hill R., 1951. Biochem. J. 50: 310.
Turowska I., Kohlmünzer S., Molik-Węgiel J., 1970. Phyto-histo-chemical Index, Medical Academy, Cracow.
Van Sumere C. F., Wolf G., Teuchy H., Kint J., 1965. J. of Chromat. 20: 48
Wood H. N., Braun A. C., 1961. Proc. Nat. Acad. Sci. 47: 1907.
Yanagawa H., Kato T., Kitahara Y., 1972. Phytochemistry 11: 1893.

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Badania niektórych metabolitów tkanki kalusowej Tecoma stans Juss.

Część III. Poszukiwania chromatograficzne irydoidów, kwasów fenolowych, terpenoidów i cukrów

Streszczenie

W tkankach kalusowych *Tecoma stans* Juss., hodowanych na zmodyfikowanej pożywce Murashige-Skooga (RT-k) oraz w liściach roślin szklarniowych, poszukiwano glikozydów irydoidowych (związki biogenetycznie związane z alkaloidami monoterpenowymi, występującymi w *Tecoma stans*) a także kwasów fenolowych, które wchodzą w połączenia estrowe z w/w glikozydami. W wyciągach, sporządzonych wg metody Trim-Hilla, nie stwierdzono obecności glikozydów irydoidowych w tkankach kalusowych i w liściach. Kwasy fenolowe oznaczono chromatograficznie (TLC) w wyciągach: przygotowanych metodą Bate-Smitha. W tkankach kalusowych znaleziono kwasy: chlorogenowe, kawowy, ferulowy, synapowy, wanilinowy i o-kumarowy, w liściach wystąpił jeszcze kwas p-kumarowy, nie stwierdzono natomiast kwasu synapowego.

Ponadto, dla pełniejszego porównania metabolizmu tkanek hodowanych *in vitro* i *in vivo*, przebadano zawartość steroli, trójterpenów i wolnych cukrów. Sterole i trójterpeny oznaczono w kwaśnych wyciągach chloroformowych z tkanek wysuszonych. W tkankach kalusowych stwierdzono, znacznie wyższą niż w liściach, zawartość β-sytosterolu. W liściach natomiast przeważają związki trójterpenowe, stwierdzono α- i β-amyrynę, kwas ursolowy i 1 nie zidentyfikowany, podczas gdy w tkankach kalusowych kwas ursolowy (większe ilości), a kwas oleanolowy oraz α-amyrynę w śladach.

Cukry oznaczono w wyciągu etanolowym ze świeżych tkanek. W tkankach kalusowych zaznaczyła się przewaga cukrów prostych. Zidentyfikowano: glukozę, fruktozę, ksylozę i sacharozę. W liściach zaobserwowano natomiast więcej wielocukrów, obok glukozy i fruktozy stwierdzono sacharozę, maltozę, rafinozę i stachiozę. Ponadto w tym wyciągu wystąpił mezo-inozytol, w znacznie większej ilości w tkankach kalusowych (0.8%), niż w liściach.

Stwierdzono, że tkanka kalusowa *Tecoma stans* Juss. jest zdolna do biosyntezy wielu metabolitów pierwotnych i wtórnych, charakterystycznych dla rośliny macierzystej, zaznaczyły się jednak różnice pomiędzy tkanką kalusową a liściem, dotyczące zawartości i ilości niektórych związków.